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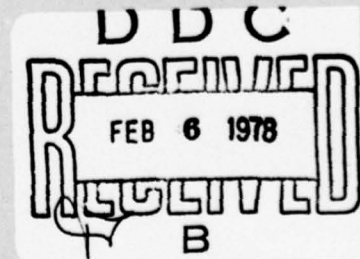
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Algae, Ultraviolet Light, and the Production of Trace Gases

P. J. HANNAN, R. A. LAMONTAGNE, J. W. SWINNERTON and C. PATOUILLET
Ocean Sciences Division

December 1977

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ALGAE, ULTRAVIOLET LIGHT, AND THE PRODUCTION OF TRACE GASES

INTRODUCTION

One of the interesting discoveries in the early 70's was that the oceans were a source of carbon monoxide (1). During the research on this phenomenon, data was also obtained on the $C_1 - C_4$ hydrocarbons; the origin and fate of these gases are of interest for several reasons, but primarily in the identification of petrogenic and biogenic sources.

The literature contains numerous references to the production of CO by microorganisms. Troxler and Dokos (2) found that CO and phyco-bilins were formed in equimolar amounts by five blue-green and one red algae. Loewus and Delwiche (3) reported that fresh tissues of algae from the California coast evolved CO; further investigation revealed that heat-treated tissues also evolved CO so long as O_2 was available, the interpretation being that CO formation was not the result of enzyme activity. Gafford and Croft (4) studied the production of CO by Anacystis and found that it was most pronounced during the stationary phase of growth. Larger aquatic organisms have been known to produce CO in substantial amounts; Pickwell et al (5) found concentrations as high as 94% in individual floats of siphonophores, and Wittenberg (6) reported CO to be a constituent in the floats of Physalia. Wilks (7) was interested in the possible production of noxious gases from the action of intense sunlight on materials within space capsules during interplanetary flights and found that CO was evolved from any polymeric material tested whether of natural or synthetic types. Wilson et al (8) demonstrated CO production by the dissolved organic carbon from cell-free algal cultures which had been exposed to fluorescent light.

The prime reason for the research described in this report was the concern over the anticipated increase in ultraviolet intensity from the sun, particularly in the 280 - 320 nm region known as UV-B, resulting from the depletion of atmospheric ozone. Does UV-B promote the production of CO, and perhaps other gases, by the algae which are ubiquitous in natural waters? Nachtwey (9) showed that solar UV-B can kill organisms, and in terms of sunlight the LD_{50} for algae was about 2 hours (10). Steeman-Nielsen (11) found an enhanced photosynthesis rate in a natural algal population covered with a glass plate compared to another culture without the plate; in both cases a black nylon net was used to reduce the sunlight to an intensity favorable for photosynthesis. He attributed the increase in photosynthesis to a partial absorption of the UV by the glass cover. Halldal (12) reviewed the UV action spectrum for algae and concluded that the region between 310 and 390 nm affected them generally in the same manner as visible

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light, but that below 310 nm biological activity was retarded. Lesions induced in deoxyribonucleic acid systems by far-UV have a damaging effect after one or two cell divisions.

Contrary to popular belief, UV does penetrate significantly into water as shown by the family of curves in Figure 1. These were calculated on the basis of sunlight at 90° incidence, with an ozone concentration of 0.32 atm. cm (13), and by means of absorption coefficients obtained by Dawson and Hulburt (14). Although these coefficients were derived over 40 years ago with equipment now considered antique, their validity is still recognized and the design of the experimentation devised for their measurement was praised in a recent publication (15).

The penetration of UV-B into water varies with the intensity, the angle of incidence, the concentrations of organic compounds and phytoplankton, and perhaps other factors. Jerlov (16) calculated the absorption at 310 nm to be 14-20% per meter for oceanic waters and 93-100% for coastal waters. Data from Figure 1 indicate that the absorption at 310 nm would be 41% per meter, and that 50% of the incident intensity would reach 1.31 meters. In either assessment it can be assumed that phytoplankton in surface waters are exposed to significant UV intensities, and the premise that UV affects CO production merits investigation. This UV effect is important in shallow areas which are the nursery grounds for larval forms of sea life.

EXPERIMENTAL

Organisms and Culture Medium: Phaeodactylum tricornutum Bohlin was the principal organism but Chaetoceros galvestonensis and Cyclotella nana were also used in this study. For growth of the stock cultures the f/2 medium of Guillard and Ryther (17) was used. Cells were centrifuged and washed with 3.5% NaCl solution before being made into a slurry with this saline solution; following a chlorophyll analysis of the slurry the desired amounts were added to a more dilute culture medium (f/24) for the growth experiments. The inocula ranged from 0.009 to 0.039 mcg chlorophyll/ml depending on the purposes of the experiment. The diluent in all cases was synthetic seawater prepared from distilled water and C.P. reagents according to the formula of Lyman and Fleming (18); the formula was modified slightly to include an additional 0.144 gm NaHCO₃ per liter since collateral tests indicated a deficiency of dissolved CO₂.

Exposure Conditions: The experiments were of two types: a) Those in which a dilute inoculum was allowed to grow for either one or two days on the roof of the building while partially shielded from full sunlight by appropriate screening, or b) Those in which cultures were grown to high chlorophyll concentrations under fluorescent light and then transferred to the roof to be exposed without shielding.

The rooftop experiments were performed with the equipment shown in Figure 2. The plastic tray contained water, 1.75 cm deep, maintained at 23°C which was circulated from a thermostatted bath containing cooling and heating elements. Each flask or bottle contained a magnetic stirring bar which was activated by a magnetic rotor in the unit beneath the tray.

To reduce the sunlight to tolerable intensities for the cultures, aluminum screen (324 mesh/in², or 50 mesh/cm²) was used in 1- to 3- thicknesses. To reduce the UV component, 0.005" mylar (0.0127 cm) film was wrapped around the appropriate flasks. The transmission of mylar as a function of wave-length is shown in Figure 3.

These experiments were carried out during the winter months, and during some of the night time exposures a temperature probe in a control flask registered as low as 7°C. During the daylight hours the temperature within the flask was approximately 20°C. A greater depth of water in the tray would have minimized the temperature excursions but the intent of this procedure was to exclude as much as possible the UV absorption by the water.

One liter quartz and pyrex Erlenmeyer flasks, and 500 ml pyrex bottles, were the test vessels. In all cases the vessels were filled to overflowing before being stoppered in order to avoid any free gas phase. For studies of this type quartz is the most suitable material because of its UV transmission but only two quartz flasks were on hand, hence the use of pyrex. While pyrex absorbs some of the UV it still transmits a large fraction of the incident intensity.

Light Intensity Measurements: Periodic determinations of sunlight intensity were made with a Lambda meter equipped with various sensors. At the time of these experiments there was no UV-B meter available, but subsequently a plot (Figure 4) of UV-B intensity vs. visible light intensity was made on a clear day. Estimates of incident UV-B data from this graph are discussed later. For the experiments conducted in cloudy weather it would be invalid to attempt an estimate in this way.

Determination of Trace Gases: The hydrocarbon and CO measurements were made by gas chromatography. In this technique, the dissolved gases are first stripped from solution by purging with helium, and are then concentrated in cold traps containing appropriate absorbents; they are subsequently released by an increase in temperature and swept into the gas chromatograph by a second stream of helium carrier gas. With this method, sample size is not restricted, and very dilute solutions may be analyzed.

Two cold traps at -77°C were used in series. In the first, activated alumina was used to trap all hydrocarbons except methane and CO; in the second, a mixture of activated charcoal and molecular sieve was used to trap methane and CO. When the stripping was complete, in about 12 minutes, the traps were isolated by closure of appropriate valves, and their temperature was raised to approximately 90°C. Helium was then used to strip each absorbent, in turn, of the absorbed gases,

and to carry these gases into the chromatograph for further separation and analysis. A four-foot column containing activated alumina with 10% Nujol was used to separate low-molecular-weight hydrocarbons other than methane. Methane was separated from CO on a molecular sieve column. A schematic diagram of the apparatus and results obtained with it are presented elsewhere (19,20).

For the light hydrocarbons, the absolute sensitivity of the method is approximately 2×10^{-12} mol. On the basis of 1-liter water samples, this sensitivity corresponds to 5×10^{-8} ml of dissolved gas at standard temperature and pressure (STP) per liter of seawater. At this lower limit, the precision of the method, on the basis of replicate measurements under laboratory conditions, is $\pm 1.0\%$. The chromatograph was calibrated with a gas mixture containing known amounts of hydrocarbons in question.

RESULTS

Gas Production with Reduced Light Intensity: Data in Table 1 summarizes the results of two experiments in which dilute *Phaeodactylum* cultures partially shielded by aluminum screens were grown in the sun for two days. During most of the exposure time the sunlight intensity was extremely high but the air temperature was below freezing, therefore the temperature inside the flasks dropped to as low as 7°C at night despite the 23°C temperature of the water bath. The effect of mylar in each case was to increase growth and to reduce CO production compared to the cultures in quartz flasks. Also the inhibitory effect of higher light intensity on growth was apparent; in the second experiment the final chlorophyll concentration in the quartz flask exposed to a peak of 700 W/M^2 was less than half that in the 350 W/M^2 exposure (0.043 vs. .092 mcg/ml). The optimum light intensity for growth of this organism is only about 20 W/M^2 .

There was clear evidence also that CO production increased with light intensity despite the lower biomass present. Variations in CH_4 concentration were minimal but the other hydrocarbons increased with light intensity in a fashion similar to CO.

The culture grown under 20 W/M^2 light intensity in the laboratory had the most biomass by far but its production of trace gases was generally lower than those cultures grown in the sun.

Diurnal Effect on Gas Production by Cultures Grown Under Favorable Light Intensities:

Only one screen was used to cover the flasks in this experiment (Table 2) compared to the use of 1 and 3 screens previously, but the light intensities encountered were low because the weather was extremely cloudy. Cultures were exposed in both quartz and pyrex, the latter covered with mylar; analyses were made after 1 day of growth, and after overnight darkness following the outdoor exposure.

As before, there was more growth in the mylar covered pyrex flasks than in the quartz but the disparity in the CO production was less pronounced. With quartz the CO reading was 16 (units omitted) while with the culture protected from UV-B the CO was only 11, both values being considerably less than most of those from the preceding experiments characterized by higher light intensities. The important point was that each of the cultures lost nearly all the CO overnight indicating that the algal cells had the property of removing CO.

Diurnal Effect on Gas Production by Dense Cultures Exposed to Strong Sunlight:

The previous experiments were designed to show the trace gas production by cultures actively growing in reduced sunlight for one or two days. The purpose of this experiment (Table 3) was to explore the effect of intense sunlight on gas production by dense cultures of algae. Three organisms (Phaeodactylum tricornutum, Chaetoceros galves-tonensis, and Cyclotella nana) were grown for 2 days under fluorescent light in media fortified with higher concentrations of nutrients than previously, including a doubling of NaHCO_3 to ensure adequate CO_2 supply and to buffer the culture against abnormal pH values. They were then placed on the roof, without shielding by either mylar or aluminum, and exposed to bright sunlight. Gas analyses were made after 8 hours in the sun and then the next morning before sunrise.

Judging from the lack of fluorescence in all the cultures after 8 hours in the sun there probably was no chlorophyll remaining although the biomass at the start of the experiment was very high, approximately 0.25 mcg chloro./ml.

A comparison between quartz and pyrex was made with the Phaeodactylum cultures, and once again the CO production was higher with quartz than with pyrex. Overnight darkness reduced the CO content of each culture but only relatively slightly, probably because the algal cells were damaged by such a high light intensity.

Increases in CH_4 and other hydrocarbons were noted in all cultures also, but there was no appreciable change between these components from the late afternoon of one day and the early morning of the next.

Investigation of Effect of Light Intensity on Dense Culture of Bacteria-Free Cyclotella:

In all previous experiments the cultures were prepared with normal care but not under sterile conditions. The purpose of this experiment was to determine whether sterile cultures would give results similar to those in which bacteria might be present. All flasks were autoclaved, then filled with the test culture (Cyclotella nana) under aseptic conditions, and samples were taken for bacterial study when the flasks were opened for gas analyses. The culture medium was the dilute f/24 solution, the same as in all other experiments except the one just prior to this. The cultures were allowed to grow for 2 days

under fluorescent light to build up a high biomass before being moved into the sun. The reason for the choice of Cyclotella nana was that in the previous experiment there had been an indication of large butene production by this organism and one of the purposes of this experiment was to check the reproducibility of this symptom. In light of the results obtained it appears that the previously high butene production was an artifact.

Sufficient flasks were used to permit the study of diurnal effects and also the effects of two light intensities (2 screens vs 0 screens). Once again the sunlight was unusually high throughout the whole day of exposure. Data in Table 4 shows that CO production was fostered by high light intensity. With 950 W/M^2 the CO reading was 347 whereas with 475 W/M^2 it was only 208. Furthermore it can be seen that the high light intensity, even with the screening of UV-B by mylar, caused almost as much CO production as that taking place in the absence of mylar. There was a notable difference, however, in the diurnal effect; the cells shielded by mylar contained less CO after a night of darkness than did the others, indicating that some vestige of metabolic activity remained whereas those receiving the full solar intensity showed no diminution of CO overnight.

Monitoring of the cultures for bacterial growth indicated that, in fact, the cultures were sterile. Furthermore comparisons with the results of the previous experiments indicated that the sterile conditions had not caused any notable changes, indicating that bacteria probably had not been significant in these experiments.

DISCUSSION

When cultures containing low concentrations of algae were exposed to low intensity sunlight their production of trace gases was minimal. In experiments summarized in Tables 1 and 2 the highest CO concentration found was 50 (units omitted in this discussion) while the combination of high light intensity and high biomass gave CO concentrations over 300 (Table 4). Shielding of cultures by mylar resulted in increased algal growth but lower CO production so long as the light intensity was not at a maximum. It is difficult to quantify these statements because of the limited number of experiments conducted thus far and also because of the unpredictability of the sunlight on any given day.

In view of the burgeoning interest in the effects of UV-B on biological systems, a plot is shown in Figure 4 of light intensity vs Sunburn Units as determined by a Sunburn Ultraviolet Meter (Solar Light Co., Philadelphia). This meter was not available during the course of these experiments but it was used subsequently to construct the plot shown on a day when there were practically no clouds. Except for the experiment summarized in Table 2, when clouds completely dominated the sky, the curve can be used to make an estimate of the Sunburn Units experienced by each culture in this study.

It is interesting to consider the effects of the extreme conditions imposed on the cultures. With only 55 W/M^2 , which represents an extremely low UV intensity, there still was a discernible difference between the cells exposed in quartz and those protected by mylar at least insofar as CO production was concerned. At 950 W/M^2 (Table 4) there was no significant difference between the CO production of cultures protected by mylar and those that were not. When the intensity was 475 W/M^2 the mylar covered culture produced only 43% of the CO in the flask without this protection. All these flasks were pyrex.

Mention should be made of two factors in this study concerning light intensity. First, it is impossible to characterize the solar conditions by a single number representing the peak light intensity, therefore it is gratifying to see that trends in the relationship between gas production and light intensity were so apparent. In order to make the data easily assimilable, no correction for the reduction of visible light by mylar was shown but it amounted to approximately 15% of the incident light. Also no attempt was made to assess the reflection of light by the flasks.

Evidence that UV-B affects metabolism can be seen in the diurnal studies made. Healthy cells reduced CO concentrations during darkness as shown in Table 2 where the CO concentration in the early morning was close to zero; they had been exposed to only low light intensities the day previous. By contrast the 950 W/M^2 flux produced very high CO concentrations by late afternoon, and there was no reduction of the CO during the night. Perhaps either the high visible light intensity, or the high UV, alone could have inactivated the cells but this is not known at this writing. Mylar absorbs most of the UV-B but does allow a significant fraction of the higher UV wavelengths to pass through, and these in concert with the visible may have been the cause of the decreased metabolic activity of the cells. In any case, it appears that a large production of CO may be an indicator of stressful conditions for the algae.

The formation of C_1 to C_4 hydrocarbons is less affected by UV and visible light than the formation of CO. Conditions fostering high CO concentrations also promote hydrocarbon formation but to a lesser extent. Certainly there is less of a change in their concentrations resulting from the mylar covering, and from darkness, than occur with CO. It would be dangerous to assume, of course, that this slight hydrocarbon production has no ecological significance because aquatic organisms are known to respond to extremely low concentrations of certain compounds. Concentrations of CH_4 and other light hydrocarbons attained in these rooftop exposures are similar to baseline levels in the ocean though the biomass concentrations here are much higher. For example, the lowest inoculum used in these experiments was 0.009 microgram chlorophyll/ml or $9 \text{ mg chlorophyll/M}^3$ in the more conventional terms of the oceanographer, corresponding to fairly heavy concentrations of plankton. Concentrations of hydrocarbons in natural aquatic environments can exceed those found in these studies.

Nonvolatile hydrocarbons, C_9 to C_{31} , in Green Lake, Ontario, amounted to 1.6 parts per billion (22) whereas the 10×10^{-5} ml per liter concentration of CH_4 characterizing certain cultures in the present study corresponds to only 0.07 ppb on a weight basis.

The role of bacteria in the formation and removal of trace gases in these experiments is not really known. In the experiment summarized in Table 4, when Cyclotella nana was the test alga and bacteria were rigorously excluded, the concentrations of CO and $C_2 - C_4$ hydrocarbons were extremely high but the light intensity imposed was also a maximum. While it is not possible to make direct comparisons between the Cyclotella in this experiment and that in Table 3, because of differences in biomass and light intensity, the indications are that the trace gases increased with the exclusion of bacteria. In future research the role of bacteria will receive increased attention.

There are literature references to the formation and removal of CO by photo-synthetic organisms in addition to those cited in the Introduction to this report. Chappelle (23) described the uptake of CO by green algae and its consequent oxidation to CO_2 ; this oxidation takes place in the dark but the rate is slower than in the light, also the rate is oxygen-dependent. Bidwell and Fraser (24) describe the uptake of CO by bean leaves, the product being CO_2 in the dark and serine and sucrose in the light. Chapman and Tocher (25) found that pneumatocysts of Nereocystis produce CO according to the concentration of oxygen in the ambient gas, there being none formed in a nitrogen atmosphere. CO can be removed from the atmosphere by anaerobic bacteria in the soil according to an account of Schnellen's work (26) by Jaffe (27). By the action of Methanosarcina barkerii it can be converted to either CH_4 or CO depending upon the availability of H_2 (28).

The matter of injury to plant systems by UV is of concern to those engaged in agriculture research. Ambler et al (29) reported that 10-day old cotton seedlings irradiated continuously in the greenhouse for 6 hours each day for 14 days showed damage. Krizek (30) studied the response of seeds and found that after 6 days exposure to UV-B there was abnormal seedling growth. How much effect there is on the growth potential of algae by UV-B must await further study. Mention has already been made of the finding by Steeman-Nielsen that UV inhibits the photosynthesis of a natural phytoplankton population, and the extreme conditions imposed in the experiments summarized in Tables 3 and 4 of this study obviously had lethal effects on the algae as shown by their lack of fluorescence. An understanding of the importance of UV-B as a factor in natural algal populations must await the determination of the various rate processes.

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TABLE I
GAS PRODUCTION WITH EXPOSURE TO REDUCED SUNLIGHT

Conditions	Peak Intensity W/M ²	Mylar	Exposure Time	Chlorophyll µg/ml	X 10 ⁻⁵ ml/l		X 10 ⁻⁶ ml/l							
					CH ₄	CO	C ₂ H ₆	C ₂ H ₄	C ₃ H ₈	C ₃ H ₆	C ₄ H ₁₀	C ₄ H ₈	C ₄ H ₆	C ₄ H ₄
Expt. #1 Control	-	No	At inocu- lation	.039	7.5	2.1	t	.46	t	t	t	t	t	t
Expt. #1 Fluor light	20	No	2 days	.232	11.0	22.4	.5	.90	.20	.30	t	.45		
Expt. #1 Roof; Quartz	300	No	2 days	.105	9.0	25.5	.5	2.0	1.1	2.4	-	-		
Expt. #1 Roof; Pyrex	300	Yes	2 days	.143	8.5	10.1	.4	1.1	2.0	1.1	-	2.3		
Expt. #2 Roof; Quartz	350	No	2 days	.092	8.7	27.8	.9	2.7	2.7	2.1	1.8	11.6		
Expt. #2 Roof; Pyrex	350	Yes	2 days	.117	8.6	23.0	1.2	2.0	4.0	Sample lost				
Expt. #2 Roof; Quartz	700	No	2 days	.043	9.8	50.0	1.2	5.3	2.7	5.2	6.2	Sample lost		
Expt. #2 Roof; Pyrex	700	Yes	2 days	.093	Sample lost		1.2	2.8	2.7	3.2	1.6	12.1		

TABLE 2
DIURNAL EFFECT ON GAS PRODUCTION BY PHAEODACTYLUM; CULTURES GROWN ON ROOF WITH LIGHT
INTENSITIES FAVORABLE TO GROWTH

Conditions	Peak Intensity W/M ²	Mylar	Exposure Time	Chlorophyll µg/ml	X 10 ⁻⁵ ml/l		X 10 ⁻⁶ ml/l									
					CH ₄	CO	C ₂ H ₆	C ₂ H ₄	C ₂ H ₂	C ₃ H ₈	C ₃ H ₆	C ₄ H ₁₀	C ₄ H ₈	C ₄ H ₆	C ₄ H ₄	C ₄ H ₂
Control	-	-	-	.016	6.1	1.5	.6	.3	.4	.2	.3	.7				
Quartz	55	No	1 day	.067	8.4	16.0	.6	2.2	.4	1.9	-	6.7				
Quartz	55	No	1 day + night	.077	8.1	4.4	.6	2.1	.4	2.1	.2	6.8				
Pyrex	55	Yes	1 day	.084	8.6	11.0	.6	1.3	.2	1.5	-	4.8				
Pyrex	55	Yes	1 day + night	.105	8.0	1.9	.6	1.4	.3	1.3	-	4.7				

TABLE 3 •
EFFECT OF STRONG SUNLIGHT AND OVERNIGHT DARKNESS ON DENSE CULTURES OF PHAEODACTYLUM,
CHAETOCEROS, AND CYCLOTETLLA

Organism; Conditions	Peak Intensity W/M ²	Mylar	Exposure Time	Estimated Chlorophyll µg/ml	X 10 ⁻⁵ ml/l					X 10 ⁻⁶ ml/l				
					CH ₄	CO	C ^H ₂	C ^H ₂	C ^H ₄	C ^H ₂	C ^H ₃	C ^H ₃	C ^H ₄	C ^H ₈
Phaeo; control	-	-	At inocu- lation	.010	16.3	2.3	2.10	1	82	.47	.04	-	-	1.5
Phaeo; pyrex	900	No	8 hrs sun		21.4	99.2	5.00	22.6	6.00	12.1	-	-	-	15.0
Phaeo; pyrex	900	No	8 hrs sun + night		23.6	83.7	5.1	23.5	6.79	14.9	-	-	-	18.0
Phaeo; quartz	900	No	8 hrs sun		20.1	116.8	5.14	24.6	5.8	13.2	-	-	-	-
Phaeo; quartz	900	No	8 hrs sun + night		20.1	71.5	5.01	25.4	6.3	15.0	-	-	-	19.0
Chaetoceros; pyrex	900	No	8 hrs sun		24.0	71.2	5.52	22.7	6.4	13.4	-	-	-	14.2
Chaetoceros; pyrex	900	No	8 hrs sun + night		23.8	64.2	5.00	21.3	5.8	12.7	-	-	-	18.6
Cyclotella; pyrex	900	No	8 hrs sun		19.0	100.6	5.00	21.7	6.0	15.1	-	-	-	35.6
Cyclotella; pyrex	900	No	8 hrs sun + night		19.5	59.2	5.61	23.0	5.72	15.0	-	-	-	Sample lost

Chlorophyll essentially zero, but
biomass equivalent to chlorophyll
content of approximately .25
µg/ml

TABLE 4
EFFECT OF LIGHT INTENSITY AND OVERNIGHT DARKNESS ON BACTERIA-FREE CYCLOTETRA

Conditions	Peak Intensity W/M ²	Mylar	Exposure Time	Estimated Chlorophyll μg/ml	X 10 ⁻⁵ ml/l									
					CH ₄	CO	C ₂ H ₆	C ₂ H ₄	C ₃ H ₈	C ₃ H ₆	C ₄ H ₁₀	C ₄ H ₈		
Pyrex	20 (Fluor)	No	0	.139	16.3	33.3	2.7	3.1	.96	1.3	-	3.1		
"	20 (Fluor)	No	After 1 day in dark	.170	16.1	41.9	1.54	4.3	.58	1.6	-	3.0		
"	475	No	8 hrs sun	.010	19.9	208.0	5.4	15.0	5.4	8.7	-	11.6		
"	475	Yes	8 hrs sun	.042	25.7	90.0	.97	2.7	.45	4.4	-	7.4		
"	950	No	8 hrs sun	0	18.4	347.0	8.55	23.8	6.5	17.0	-	16.1		
"	950	No	8 hrs sun + night	0	19.3	356.0	13.0	36.0	8.3	28.0	-	23.2		
"	950	Yes	8 hrs sun	0	18.9	335.0	7.98	22.2	6.4	18.4	-	16.2		
"	950	Yes	8 hrs sun + night	0	19.1	267.0	7.8	27.3	7.3	12.0	-	20.2		

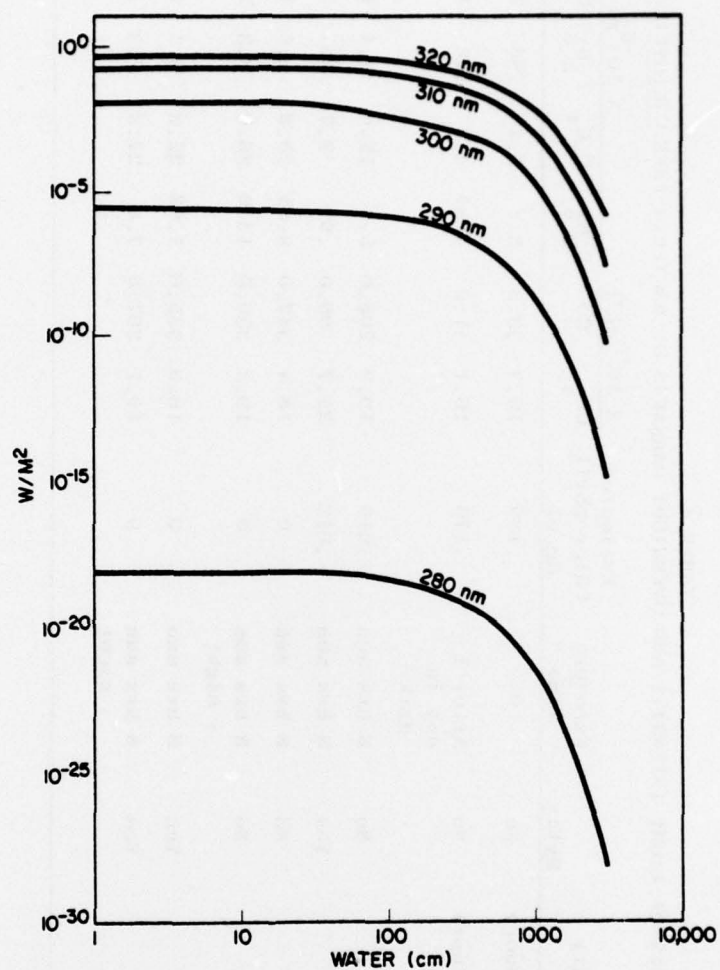


Fig. 1 — Penetration of UV-B into distilled water, calculated on the basis of incident light at 90° and the ozone concentration at 0.32 atm. cm.

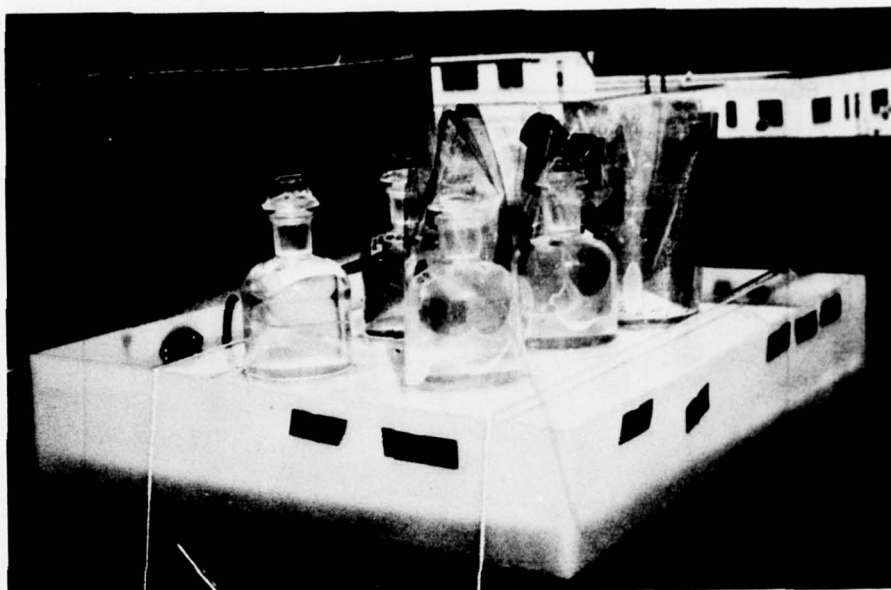


Fig. 2 — Picture of rooftop exposure equipment including constant temperature bath, pyrex bottles, and shields of mylar and aluminum screen

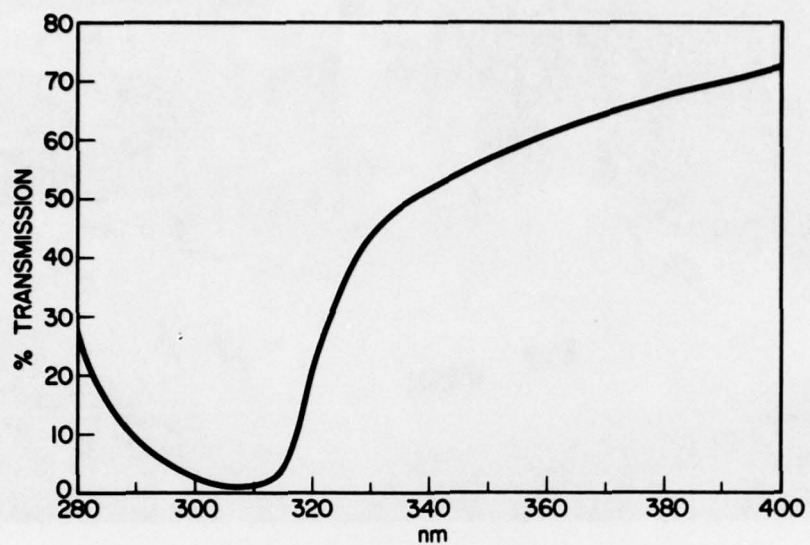


Fig. 3 — Percent transmission of UV by 0.005" mylar

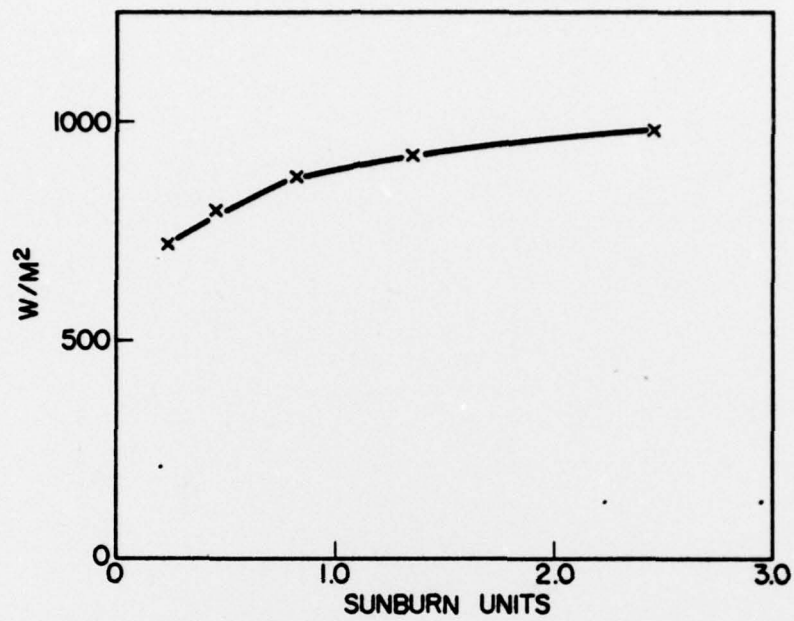


Fig. 4 — Plot of Sunburn Units vs visible light intensity on clear day